

Obligatory separation of hormone binding and biological response curves in systems dependent upon secondary mediators of hormone action

(cyclic AMP/spare receptors)

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ABSTRACT A mathematical model is presented that describes the effects of hormone concentration on receptor saturation and biological response in systems dependent upon the generation of a secondary mediator such as cyclic AMP. The analysis makes the following assumptions: (i) the binding of hormone to its receptor is a reversible, second-order reaction; (ii) the concentration of mediator that is generated is directly proportional to the number of membrane binding sites occupied by hormone; and (iii) the binding of the mediator with its intracellular receptor to generate an effector complex is also second-order and results in a proportionate biological response. It follows from this treatment that the hormone concentration required for half-maximal biological response is formally lower than that required for half-maximal receptor saturation and that the difference between these two concentrations will depend upon the ratio of total mediator generated at full receptor occupancy to the dissociation constant of the mediator with its receptor. Without invoking concepts of negative cooperativity, this model offers a simple explanation for discrepancies between receptor occupancy and biological response curves that are often observed. Moreover, the mathematical form of the predicted biological response curves conforms to the shape of the response curves observed experimentally in a wide variety of systems.

The widespread availability of radiolabeled hormones has recently made possible a quantitative description of many hormone-receptor interactions. In studies of such interactions, comparisons are often made between the concentration of hormone required for half-maximal biological response (K_b), and the concentration required for half-maximal receptor occupancy (K_d). In some cases these two concentrations are similar, but in many others K_b is much smaller than K_d (1-5). Various approaches have been used to account for these latter differences. On one hand, it has been suggested that much of the observed hormone binding is either nonspecific or related to simple occupancy of hormone storage sites and that a large proportion of the measured receptors do not participate in the biological response (6). On the other hand, several models have been devised that predict a nonlinear relationship between hormone binding by functional receptors and biological response (7-10).

In this paper we present a new mathematical model that addresses the dependence of receptor occupancy and biological response upon hormone concentration. This model, based on the assumption that interaction of a hormone with its receptor results in the generation of an intracellular intermediate that in turn interacts with its own intracellular receptor to effect a

proportionate biological response, predicts differences in the concentrations required for half-maximal receptor binding and half-maximal biological response under a variety of conditions. Specifically, it leads to the conclusion that K_b must be lower than K_d whenever the hormonal response is dependent upon the generation of an intracellular mediator such as cyclic AMP (cAMP).

The noteworthy aspects of our analysis are its demonstration that discrepancies between K_b and K_d follow from the most elementary treatment, and its ability to predict the experimentally observed form of the biological response curves in many systems.

RESULTS AND DISCUSSION

Classically, interactions between a hormone, H, and its receptor, R, are quantitatively described by the Law of Mass Action expression

$$\frac{[R][H]}{[RH]} = K_d,$$

where [RH] represents the equilibrium molar concentration of the receptor-hormone complex, and K_d represents its equilibrium dissociation constant. If R_t represents the concentration of total available receptor ($R_t = [R] + [RH]$), the expression can be rewritten as

$$\frac{(R_t - [RH])[H]}{[RH]} = K_d. \quad [1]$$

[RH] is generally measured by the binding of radiolabeled hormone, and R_t is determined by extrapolating the values of [RH] obtained at different hormone concentrations to infinitely high concentrations of H.

Eq. 1 can be rearranged to yield

$$\frac{[RH]}{R_t} = \frac{[H]}{K_d + [H]}, \quad [1']$$

which gives the ratio [RH]/ R_t (the fractional occupancy of receptor by hormone) as a function of free hormone concentration. A plot of [RH]/ R_t as a function of [H] is shown in Fig. 1; the slope of the curve is given by

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Abbreviations: cAMP, cyclic AMP; hCG, human choriongonadotropin (chorionic gonadotropin).

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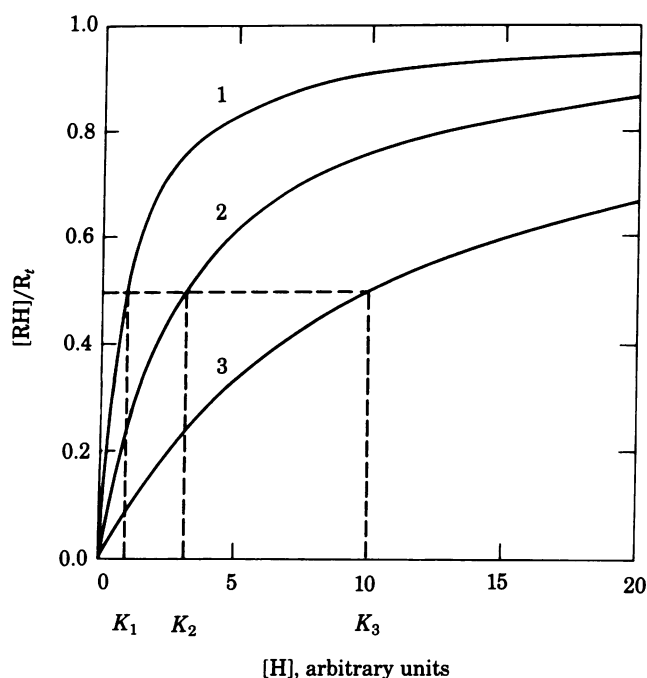


FIG. 1. Fractional receptor occupancy as a function of hormone concentration $[H]$. The concentration of hormone at 50% occupancy is equal to the dissociation constant (K) for the RH complex. The curves are drawn such that K_2 and K_3 differ from K_1 by factors of $\sqrt{10}$ and 10, respectively.

$$\frac{d([RH]/R_t)}{d[H]} = \frac{K_d}{(K_d + [H])^2}.$$

For concentrations of $H \ll K_d$, the relation between $[RH]/R_t$ and $[H]$ is linear and has a slope of $1/K_d$. Conversely, for values of $[H] \gg K_d$, $[RH]/R_t \approx 1$ (corresponding to receptor saturation), and the slope of the curve asymptotically approaches zero. Between these extremes, the slope of the curve decreases continuously from $1/K_d$ to 0; at $[H] = K_d$, $[RH]/R_t = 1/2$, and the slope is $1/4K_d$.

For convenience, Eq. 1' is frequently plotted with a logarithmic scale on the abscissa (Fig. 2); this curve has a slope given by

$$\frac{d([RH]/R_t)}{d \ln[H]} = \frac{K_d[H]}{(K_d + [H])^2}.$$

In contrast to the curve shown in Fig. 1, the semilogarithmic plot has both initial and final slopes of zero, and the slope at $[H] = K_d$ reaches a maximum of $1/4$, a value now independent of the magnitude of K_d itself. Families of curves for fractional receptor occupancy with varying values of K_d are also shown in Figs. 1 and 2. The semilogarithmic representation, when plotted parametrically in K_d , results in a family of parallel curves differing from each other only in horizontal displacement along the abscissa; the slope of each curve is a function of the ratio $[H]/K_d$. In addition to conferring geometric simplicity, the use of a logarithmic abscissa in Fig. 2 permits the simple representation of fractional receptor occupancy over a wide range of hormone concentrations.

In systems where there is direct coupling between receptor occupancy and some physiological response (e.g., for a confor-

mational change upon receptor occupancy that results in a direct change in membrane permeability), the hormone concentrations required for half-maximal receptor occupancy and half-maximal physiological response should obviously be identical. In contrast, as we shall show, discrepancies between fractional binding and fractional response are to be expected in other systems in which the hormonal response is instead dependent upon the generation of an intracellular mediator (e.g., cAMP), which must itself interact with some more distal receptor or effector mechanism (e.g., a protein kinase).

Let K be the dissociation constant for the complex AB of any soluble secondary mediator A with its own intracellular receptor B and, for simplicity, let the steady-state intracellular concentration of A that is generated be directly proportional to the concentration of membrane binding sites occupied by hormone.[§] Then,

$$\frac{[A][B]}{[AB]} = K,$$

and

$$[A] = a[RH],$$

where a is a proportionality constant and $[A]_{\max} = aR_t$ at $[RH]/R_t = 1$. If the total number of intracellular binding sites for the secondary mediator A is B_t , where $B_t = [B] + [AB]$, and if the physiological response Φ is proportional to the concentration of AB , then

$$\Phi = b[AB] = \frac{bB_t[A]}{K + [A]},$$

[§] This condition will be satisfied if the rate of synthesis of A is directly proportional to receptor occupancy and if the degradation of A displays first-order kinetics.

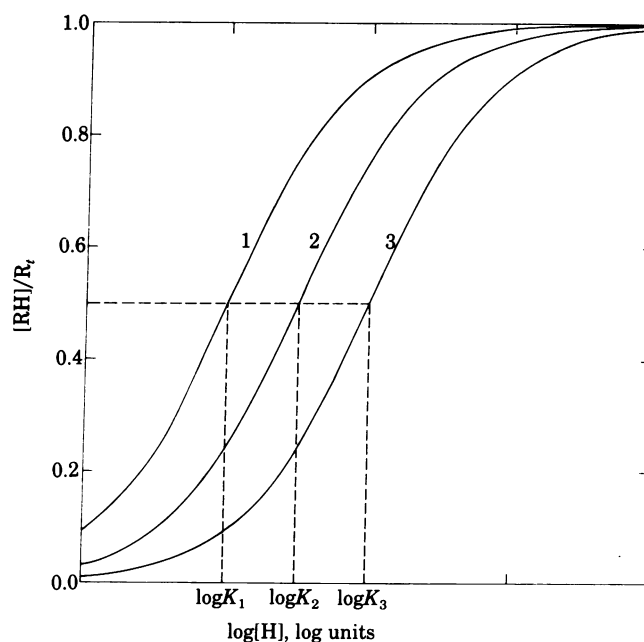


FIG. 2. Fractional receptor occupancy as a function of the logarithm of hormone concentration. The curves correspond to the same ones shown in Fig. 1.

where, similarly, b is a proportionality constant. Under such conditions it follows that

$$\Phi = \frac{abB_t R_t [H]}{KK_d + (K + aR_t)[H]}, \quad [2]$$

and, hence, at infinite hormone concentration,

$$\Phi_{\max} = \frac{abB_t R_t}{K + aR_t}.$$

The physiological response expressed as a fraction of the response at full hormone receptor occupancy will then be given by

$$\frac{\Phi}{\Phi_{\max}} = \frac{(K + aR_t)[H]}{KK_d + (K + aR_t)[H]} = \frac{[H]}{\frac{KK_d}{(K + aR_t)} + [H]} \equiv \frac{[H]}{K_\Phi + [H]},$$

which has a form identical to that for fractional receptor occupancy itself, namely

$$\frac{[RH]}{R_t} = \frac{[H]}{K_d + [H]}.$$

Thus, respective plots of $[RH]/R_t$ and Φ/Φ_{\max} versus $\log[H]$ will be parallel throughout their courses and will differ only in their position along the abscissa. Half-maximal hormone binding will occur at $[H] = K_d$, whereas the half-maximal physiological response will occur at $[H] = KK_d/(K + aR_t) \equiv K_\Phi$.

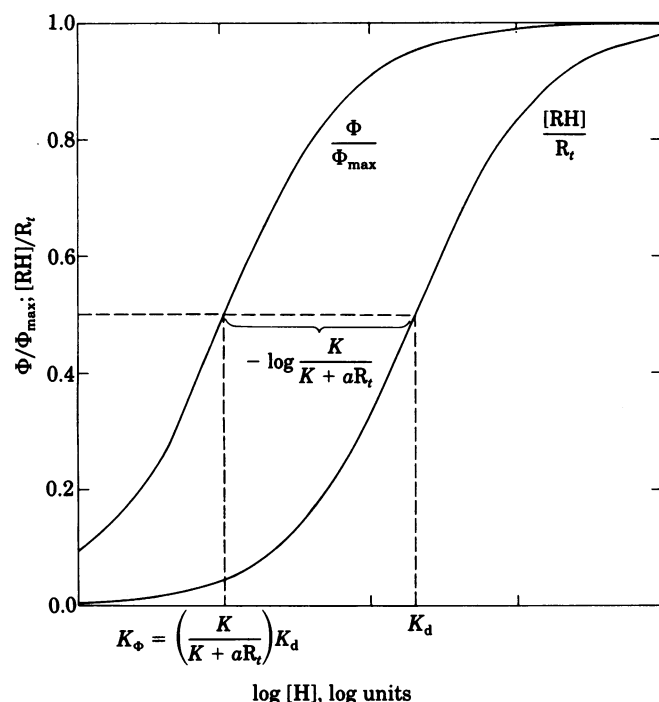


FIG. 3. Fractional receptor occupancy ($[RH]/R_t$) and fractional physiological response (Φ/Φ_{\max}) as functions of the logarithm of hormone concentration. The curves differ in their position on the abscissa by $-\log [K/(K+aR_t)]$.

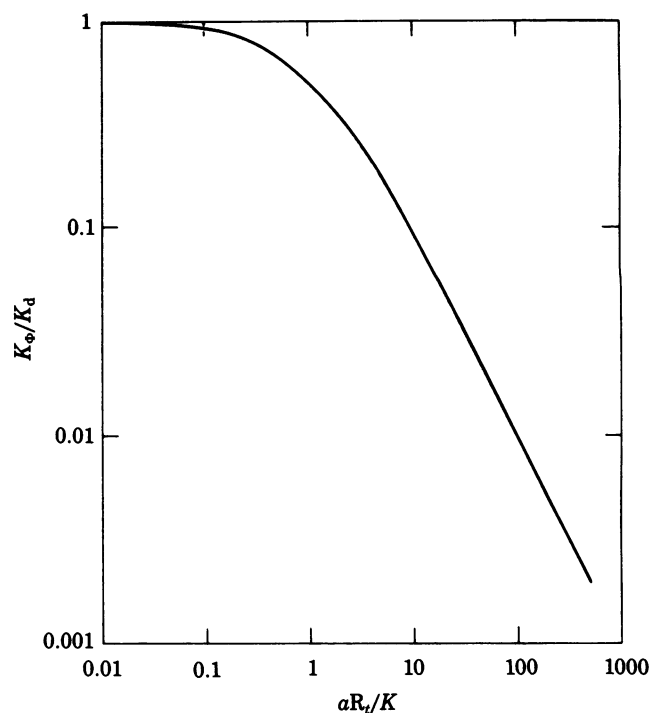


FIG. 4. K_Φ/K_d expressed as a function of aR_t . For $aR_t \ll K$, K_Φ is approximately equal to K_d , whereas for $aR_t \gg K$, K_Φ is much less than K_d . aR_t is the maximal concentration of intracellular mediator generated at full receptor occupancy; K is the dissociation constant for the complex of this mediator with its intracellular receptor.

The conclusion that follows from this treatment is that concern about discrepancies between hormone binding curves and corresponding physiologic response curves may be unwarranted. Because $K_\Phi = KK_d/(K + aR_t)$, it follows that $K_\Phi < K_d$ and, hence, that the physiologic response curve must lie "to the left" of the curve for receptor occupancy (Fig. 3). The degree of displacement of the two curves will be given by $-\log K/(K + aR_t)$ and, thus, will be determined by the ratio aR_t/K (Fig. 4). Only when $aR_t \ll K$ will the fractional physiological response curve approach the curve for fractional receptor occupancy; any other values for $aR_t \ll K$ will lead to a separation of the two curves.

It is instructive to discuss this result briefly in nonmathematical terms considering, as an example, cAMP as the intracellular mediator and a protein kinase as the molecule with which cAMP interacts to effect the physiological response. If the cAMP concentration achieved at maximal hormone receptor occupancy is much less than the dissociation constant for the cAMP-protein kinase complex, the concentration of the latter complex, like that of cAMP itself, will be very nearly proportional to hormone receptor occupancy throughout the entire range of hormone binding; in such a case, the curves for fractional physiological response and fractional receptor occupancy will be virtually superimposable. If, at the other extreme, the cAMP concentrations generated can appreciably exceed the dissociation constant for the cAMP-protein kinase complex, a point will be reached well below full hormone receptor occupancy at which the protein kinase is saturated, and a further increase in cAMP concentration will not change the biological response. In this instance, the curves for receptor occupancy

and response will be substantially displaced.[†]

One of the interesting consequences of the foregoing treatment is that the degree of displacement of the physiological response curve appears as an explicit function of total receptor number; in particular, for any given values of K and a , an increase in R_t will produce a further shift of Φ/Φ_{\max} to lower concentrations of hormone. The presence of a large number of unoccupied ("spare") receptors at concentrations of hormone in the physiological range should not be regarded as anomalous; instead, because the absolute magnitude of the biological response (Φ) at any given hormone concentration increases continuously with the total number of receptors present (Eq. 2), a large receptor number provides the cell with heightened hormonal sensitivity (1, 4).[‡]

Several other models have been developed that also can account for differences between K_Φ and K_d (7–10). In general, these analyses focus on the coupling between the receptor-hormone complex and its target effector unit (e.g., adenylate cyclase). Whereas some of these approaches bear resemblance to the treatment described here, our model is considerably more simple and requires few assumptions. Although we have not addressed the question of negative cooperativity between receptor binding and hormone concentration, which has been explicitly treated by others (7–12), it should be noted that systems involving cAMP in general do not exhibit such cooperativity and that our model accurately predicts the mathematical form of the observed biological response curves.

The above analysis assumed, for convenience, that the concentration of intracellular mediator generated is directly proportional to receptor occupancy at all concentrations of hormone. In the instance of cAMP, this relationship holds true for isoproterenol binding to turkey erythrocytes, where the curves for hormone binding and cAMP generation are superimposable and only the physiological response curve for monovalent cation transport is displaced (5). In a contrasting example, it has been shown that cAMP formation in rat Leydig cells is not directly proportional to human choriogonadotropin (hCG; human chorionic gonadotropin) binding (3, 4). Here, three separate concentration-response curves are observed, all of identical form but differing by their position along the abscissa: the half-maximal physiological response (testosterone production) occurs at the lowest hCG concentrations (≈ 0.3 pM), cAMP generation is half-maximal at intermediate levels of hCG (≈ 5 pM), and half-

maximal receptor occupancy is displaced to still higher hormone concentrations (≈ 80 pM). These findings have led some authors earlier to question whether cAMP is a necessary intermediate in the stimulation of testicular steroid production by hCG (3, 4).

An alternative explanation emerges from our analysis. With regard to the difference in dose-response curves for steroid production and cAMP generation, this result does not imply that cAMP is not the mediator but rather that considerably less than maximal cAMP concentrations are sufficient to saturate the subsequent binding reaction that leads to the biological response. The displacement between cAMP generation and hormone binding suggests that in the Leydig cell these events are not directly coupled and that an additional reaction involving the binding of some intermediate substance intervenes.^{**} Possible candidates for such an intermediate include a protease, which has been proposed to mediate the hCG stimulation of adenylate cyclase that leads to steroidogenesis in rat ovarian cells (13), or GTP, which activates adenylate cyclase in many systems (14) (see ref. 15 for review).

CONCLUSIONS

There are now many examples of biological responses that become maximal at hormone concentrations well below those required for a comparable degree of receptor saturation, adenylate cyclase activation, or cAMP production. Specific hormones for which this is the case include insulin (1, 2), hCG (3, 4), β -adrenergic agonists (5, 16), corticotropin (adrenocorticotrophic hormone) (17, 18), lutropin (luteinizing hormone) (19), glucagon (6), and vasopressin (20). The analysis presented here offers a particularly simple explanation for these observations.

Many of these same concepts can be extended with little modification to considerations of enzyme kinetics as described by the Michaelis-Menten formulation, which are formally identical to many aspects of hormone-receptor interactions. An analogous approach can be applied to sequential enzymatic reactions in which changes in intermediate substrate (or regulatory ligand) concentrations well below those corresponding to the K_m s for their respective enzyme complexes can produce maximal changes in overall reaction rate.

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[†] The displacement of K_Φ , in addition to permitting maximal changes in Φ at hormone concentrations considerably below K_d , makes it possible for these changes to be more rapidly reversible than would be the case if comparable hormone sensitivity were conferred through a reduction in K_d . In the latter instance the decrease in dissociation rate for the RH complex required for a substantial decrease in K_d would preclude a rapid reversal of physiological effect unless there exist other mechanisms to reduce [RH] (e.g., receptor "internalization").

[‡] The magnitude of this increase is obtained explicitly by differentiation of Eq. 2 to yield

$$\left(\frac{\partial \Phi}{\partial R_t}\right)_{[H]} = \frac{KabB_t[H](K_d + [H])}{[KK_d + (K + aR_t)[H]]^2}.$$

At low hormone concentrations, $(\partial \Phi / \partial R_t)_{[H]}$ is independent of R_t but directly proportional to $[H]$, and Φ increases linearly with R_t for all values of R_t . At high hormone concentrations, $(\partial \Phi / \partial R_t)_{[H]}$ becomes independent of $[H]$ and is a decreasing function of R_t ; in this latter case, Φ increases linearly with R_t only as long as R_t remains small ($R_t \ll K/a$). When $[H]$ and R_t are both large, $(\partial \Phi / \partial R_t)_{[H]}$ approaches zero.

^{**} The mathematical treatment presented earlier can be extended to include the general case in which the generation of A (mediating the ultimate physiological response) is not directly coupled to hormone receptor occupancy but requires instead the intervening generation of one or more sequential intermediates. As previously indicated, other models not requiring an additional intermediate but instead involving concepts of membrane fluidity and receptor mobility have been proposed to account for nonlinearity between hormone binding and cAMP generation.

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